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Permanent Value of Major Walter Reed's Work on Yellow Fever*

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I WISH to thank the officers of the American Public Health Association for the honor they have done me in inviting me to present a paper on Walter Reed's work in yellow fever. It is a particular pleasure for me to do this because as a junior medical officer in the medical corps of the army I had the privilege of knowing Walter Reed personally. To be sure, our relations were such as you would expect between a distinguished senior officer and a rather green lieutenant. I look back with pleasure to the acquaintance, and I shall always remember his friendly way of being helpful to the younger men in the medical corps of the army.

It was in October, 1900, at Indianapolis, Ind., that Walter Reed [1] presented a preliminary report before this Association, describing the work carried out up to that time by the army yellow fever board, which included in its membership, in addition to Walter Reed, James Carroll, Aristides Agramonte, and Jesse W. Lazear.

In this preliminary report Reed first disposed of the *Bacillus icteroides* of Sanarelli, by showing that neither in blood cultures from 18 patients nor at autopsies of 11 cases was this bacillus to be found. The second part of the paper deals with the mosquito, *Aedes aegypti* (known formerly and mentioned by Reed, as *Culex fasciatus*), as the intermediate host of the parasite of yellow fever.

Reed was fully aware of the hypothesis of Carlos Finlay [2] of Havana, but was unconvinced by his ingenious discussions. In fact, Reed states: "We believe that he has not as yet succeeded in reproducing a well-marked attack of yellow fever within the usual period of incubation of the disease, attended by albumen and jaundice, and in which all other sources of infection could be excluded."

Reed was, on the other hand, greatly influenced by the work of Surgeon Henry R. Carter,[3] U. S. Public Health Service, who studied cases of yellow fever at Orwood and Taylor, two neighborhoods in rural Mississippi. Carter observed that it was quite safe for friends to visit a house for a period of 2 or 3 weeks after the development of

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the first case of yellow fever in the house, but very dangerous to visit the house after that time. In other words, the house did not become infected until 2 to 3 weeks after the arrival of the patient in the house. Carter called this the period of extrinsic incubation, in contrast to the incubation period of the disease of 1 to 7 days in the person contracting it.

This extrinsic incubation period suggested to Reed the probability of an insect carrier, such as has been found by Sir Ronald Ross in malaria. In the preliminary note presented at Indianapolis he reported 2 positive cases of yellow fever caused by bites of the *Aedes aegypti,* with the usual period of incubation and in which other possibilities of infection could be excluded. The paper is short, concise, and clear, and will well repay the trouble of reading it again.

It is interesting that 33 years later, as one studies Reed's paper, one finds that nothing has to be changed. Much has been added to our knowledge of yellow fever, particularly since 1927, but nothing has had to be taken away from the findings and conclusions embodied in the paper; and this demonstrates, better than anything I can say, the merit and the soundness of Walter Reed's work.

After the Indianapolis meeting Reed returned to his studies in Havana, but while he was still in the United States he made a complete schedule of the experiments to be carried out. These were so arranged that each hypothetical question would be answered by a definite yes or no. This arrangement of the investigation, breaking down the problem into a number of definite hypotheses, each of which can be answered by yes or no, is called by the Germans a "Fragestellung." An examination of Reed's Fragestellung is well worth while, since it is in all respects a model.

In his second paper, read at the Pan American Medical Congress in Havana, Cuba, in February, 1901, Reed was able to present further evidence that the mosquito carried yellow fever, and under what circumstances.

He built a small camp in an isolated place, where he could control absolutely the entire personnel and prevent infection from occurring in the usual way. In this small community of 12 nonimmune persons he produced at will 2 outbreaks of yellow fever, the first of 4 cases, the second of 1; and he could limit the number of cases to the number of persons he exposed to the bites of infected *A. aegypti* mosquitoes. The proof that the *A. aegypti* was the intermediate host of the infection was clear and complete.

Following this he showed, as the second necessary step, that the disease could not be propagated in other ways. It had long been the custom of health departments to disinfect houses where yellow fever had occurred, and, of course, every article in the sick room. Quarantine officers were accustomed to disinfect ships, including the cargo and even rocks carried as ballast, and, of course, the passengers' baggage.

Reed determined to test by experimental methods the validity of the common belief that yellow fever was conveyed by fomites. He erected a small house, 14 by 20 feet, with poor ventilation and a rather dark interior but with good screening. In this he placed the soiled linen of patients who had died of yellow fever. Of all classes of fomites, bedding and clothing had always been considered the most dangerous.

In this dark, ill-ventilated room, furnished with soiled bedding and clothing, Dr. R. P. Cooke and 2 privates of the hospital corps slept every night from November 30 to December 19, 1900, a period of 20 days. The experiment was repeated with 2 other non-

immune young Americans for another period of 20 days, ending on January 10, 1901, after additional recently contaminated bedding and clothing had been added. The experiment was repeated a third time with 2 other non-immune young Americans and fresh fomites.

The total period of exposure to so-called infected fomites was 63 days. Seven non-immune persons were exposed, and none of them became infected. Reed's demonstration of the erroneousness of the time-honored doctrine of infectious fomites was immediately accepted by the world.

The third step was to repeat the mosquito work under conditions which could be controlled exactly, and for this purpose he built a second small house, called the infected mosquito building, which was divided into halves by means of a huge screen of wire mosquito netting, extending from floor to ceiling. The only difference between the two rooms was that one contained *A. aegypti* mosquitoes, which had fed on yellow fever patients in the first 3 days of their illness, and the other room did not. Into both rooms he introduced non-immune soldiers; those admitted to the mosquito room contracted yellow fever; those admitted to the mosquito-free room did not. Although the results of the first series of experiments were quite convincing, they lacked what in experimental parlance is called a control. This lack was supplied in the third series by the twin room building, where the conditions were identical on the two sides of the screen, except for the infected mosquitoes on the one side.

One other fundamental experiment was carried out at this time. Reed, Carroll, and Agramonte [4] produced 4 cases of yellow fever by the subcutaneous injection of blood from yellow fever patients in the first and second days of their illness. This direct method of transfer showed that the infectious agent was present in the blood, and that mosquitoes added nothing to it, but played a rôle similar to that of anopheles mosquitoes in malarial fever.

This made Reed's conclusions incontestable and convincing to the world, and they have remained true and incontestable to this day. In all the long history of medicine and of natural science there is no better example of the use of the experimental method than this work of Reed and his colleagues. They had shown that yellow fever is carried by *A. aegypti* and that it is not conveyed by fomites.

Reed at this time returned to Washington, but Carroll remained in Havana and studied the effect of filtered blood serum from yellow fever patients. He found that the infectious agent passed through a Berkefeld filter which held back bacteria, and that the infectious agent resembled in this respect the virus of hoof and mouth disease. This was one of the earliest observations on filter passing viruses, and Carroll's findings have been fully confirmed in the past few years.

A survey of the city of Havana had shown that *A. aegypti* was everywhere present in vast numbers, and Reed logically recommended the destruction of these mosquitoes and the protection of the sick against their bites. As a result, all fever hospitals both civil and military were screened, and an anti-mosquito campaign, inaugurated by General Gorgas, the chief medical officer in Cuba, was begun. The campaign was continued and extended, and within a year the disease in Cuba was brought under control, and ultimately eradicated. This closes the first chapter in the modern history of yellow fever.

Reed, having found the method of transmission of the disease, and having recommended a campaign against it based on mosquito control, was recalled to Washington to take up his work at the Army Medical School. As

we look back, we cannot but regret that he did not continue his studies, free from teaching duties. He might have advanced our knowledge and anticipated much of the work of the past decade. What Walter Reed lacked was an experimental animal; he was compelled to use human beings, which greatly limited his experiments. Had he been permitted to continue the yellow fever studies he might have found years ago the animal suited to his needs, and continued his contributions to our knowledge of the disease. As it was, no progress was made from 1901 to 1927.

The second chapter of yellow fever studies begins in 1927 with the discovery by Stokes, Bauer, and Hudson [5] that the Asiatic monkeys, *Macacus rhesus* and *M. sinicus,* were susceptible to yellow fever and might be used as experimental animals. This finding was the result of a long painstaking search for an experimental animal. Every possible species of West African animal including the chimpanzee was tested, and none of them was found susceptible, a rather interesting and important observation. It was not until the investigators tested the common monkey of the zoölogical garden, *Macacus rhesus* from India, that they were successful. Now that at last an experimental animal was available it became possible to take up the work where Walter Reed had dropped it. Reed's findings were confirmed and extended.

Although yellow fever in monkeys is usually fatal, nevertheless some of the monkeys that were experimentally infected survived, and they remained immune just as human beings do. Their immunity was shown, (1) by resistance to re-infection, and (2) by the fact that their blood serum would protect another monkey against a fatal dose of the virus. This suggested the possibility of a protection test. Such a test is made as follows: Along with a fatal dose of the virus is given the serum to be tested, whether of man or monkey. If the monkey survives, the protection test is positive and the donor of the serum must have had yellow fever at some time in the past. The protection test has become the basis for extensive epidemiological surveys, which have permitted later investigators to map out the distribution of the disease in Africa and South America.

As stated, West African monkeys are completely resistant to yellow fever infection. Some of the South American monkeys show evidence of infection but do not develop the symptoms or pathological lesions as completely as Asiatic monkeys. Sawyer and Frobisher [6] have shown that other animals can develop immune bodies in their serum, although there is no mortality and usually no illness. This is true of the rabbit, ferret, guinea pig, and even the hen. Pettit, Stefanopoulo, and Frasey [7] showed that immune bodies could be produced in horses, and although they were produced in quantity there was no apparent illness and, of course, no fatalities in this animal.

In 1930, Max Theiler,[8] then at Harvard, found that a characteristic encephalitis could be produced in albino mice by injecting yellow fever virus directly into the brain, and further that the mouse could be used for the protection test by injecting the serum to be tested together with the virus directly into the brain.

The mouse brain is so small that only the most minute quantities can be injected into it, and for this reason Sawyer and Lloyd [9] modified Theiler's procedure in an ingenious way. They produced a minimum injury to the brain by injecting into it 0.02 c.c. of a sterile starch solution or other irritant, and then put relatively large quantities of virus and the serum to be tested into the peritoneal cavity. The slight injury to the brain produces a point of least resistance in which the virus is implanted by the

blood stream, and under these conditions the virus increases and the usual encephalitis ensues if the serum is negative; if, however, the mice survive, it is evidence that the person furnishing the serum has at some time in the past had yellow fever. This is now the standard protection test.

Sawyer and Lloyd [9] made another important observation as to yellow fever virus in albino mice; this is, that not all races of albino mice are equally susceptible, and that satisfactory work can be done only with certain selected strains of such mice, the heredity of the mouse being of great importance.

Walter Reed showed that at summer temperatures *A. aegypti* did not become infective until about 12 days after taking yellow fever blood, and that in the colder weather of the fall the period was prolonged. Bauer and Hudson, working in Lagos, Nigeria, in West Africa, found that the extrinsic incubation period might be as short as 9 days. Davis [10] recently showed that the length of the period is affected by the temperature: at a constant temperature of 37° C. the period is only 4 days; at 36° C. it is 5 days; at 31° C. it is 6 days; at 23 to 25° C. it is 11 days; and at 21° C. it is 18 days. If the mosquitoes were kept at 18° C. they were still unable to transmit the disease after 30 days, but if they were then warmed up to summer temperature they became infective after only 6 days more. We now know that the extrinsic incubation period varies according to the temperature.

Does the virus multiply while in the mosquito? Davis, Frobisher, and Lloyd [11] have titrated the virus in the mosquito and have concluded that the virus does not multiply in the insect, but rather decreases in quantity with time, although enough remains so that the mosquito continues infectious throughout life. This is in marked contrast to malaria, in which disease, as we know, the parasite multiplies enormously in the mosquito. Davis and Shannon [13] showed that the virus of yellow fever dies with the mosquito and is not transferred to other adults with the ova.

Reed attempted to transmit the disease with one other mosquito, *C. pungens,* but failed. Since we have had experimental animals many other mosquitoes have been studied, and under laboratory conditions no less than 13 new vectors have been found, a result which would not have surprised Reed. He wrote that the subject needed further study. In nature, however, only 2 new vectors have been encountered. In Espirito Santo in Brazil, Soper [12] found a small epidemic of yellow fever going on in the absence of *A. aegypti,* but there were numerous *Aedes fluviatilis* and *scapularis. A. aegypti,* however, without doubt, remains the important vector.

PATHOLOGY OF YELLOW FEVER

Councilman,[14] in 1890, studied yellow fever tissues at the request of Sternberg and described accurately the scattered acidophilic hyaline degeneration of parenchyma cells and parts of cells in necrotic areas of the liver, which we now know is characteristic of yellow fever.

Rocha Lima [15] of Brazil first drew attention to the midzonal distribution of the necrosis of the parenchymatous cells of the liver lobule. The cells about the central vein and along the periphery are rarely attacked.

Klotz and Belt [16] describe the changes in the liver as a non-inflammatory necrosis of the parenchyma, unaccompanied by collapse of the tissue or by interstitial hemorrhage, but with more or less cloudy swelling and fatty degeneration. If recovery occurs, there follows regeneration of the parenchyma without fibrosis. Hudson [17] finds the same sequence of events in monkey livers.

Torres [18] of Rio de Janeiro demonstrated the presence of intranuclear cell inclusions in the livers of monkeys. This important observation was confirmed by Cowdry and Kitchen [19] and by Klotz and Belt.[20] While the inclusion bodies are found most easily in the livers of monkeys and the brains of mice, they nevertheless can be found in the human liver by careful search.

VACCINATION

The number of accidental laboratory infections was, considering the limited number of workers, very large. We have records of 33 cases with 6 deaths, and the danger was so great that it was imperative that some method of protection be found.

It had been noted that monkeys which received immune serum along with the virus were protected against illness and death. In May, 1931, Sawyer, Kitchen, and Lloyd,[21] after much preliminary work on monkeys, began vaccinating the laboratory personnel with a serum-virus mixture. Up to the present time, over 100 persons have been immunized without any untoward results, and there has been no further illness among the laboratory personnel.

The present vaccine is therefore successful, but it is cumbersome and costly, and many studies are under way with the object of making it simpler. Some of these studies are quite promising. If the vaccine can be simplified it will be of great help in fighting the disease in infected regions. For example, the entire air personnel could be protected.

DISTRIBUTION OF YELLOW FEVER

The present wide distribution of yellow fever is important and is only now becoming known. So far as we are aware there is no longer any in North or Central America. In South America it is found in northern Brazil, including the Amazon valley, and on the eastern slopes of the Andes in Bolivia. There is also a small but persistent focus in the interior of Colombia. Although the infected area in South America is extensive, it is constantly diminishing. There are, for example, no longer any foci on the Pacific coast, and it is not unreasonable to expect that if the present campaign is carried out actively for a long enough time the Americas may be freed from the disease.

In Africa, however, the situation is quite different. The area involved is very extensive and the population is huge; in Nigeria alone, there are 20 million blacks living under the most primitive conditions. In Senegal, the Belgian Congo, French Equatorial Africa, Uganda, and the southern end of the Anglo-Egyptian Soudan, are millions of primitive Africans. It will be a long time before the state of civilization and sanitation will make it possible to fight the disease effectively in this region.

A. aegypti mosquitoes can be controlled in the coast towns, however, and the transportation personnel can be vaccinated. Dr. Henry R. Carter believed that West Africa is the real home of yellow fever and that the disease was brought to the Americas by slave ships.

The endemic areas in South America and West Africa were quite harmless to Europe and America until new methods of travel were perfected. Now as new regions are opened up by automobile travel, the disease spreads, and as vast regions are brought close together by the airplane we are again in danger of having yellow fever carried to the Mediterranean and to our own shores.

It behooves us therefore to continue to pursue the study of the disease and its habits of spread, with all the energy possible; to devise better methods of protection than we have had; to develop the practice of vaccination still further; and so to carry on the great work which Walter Reed began in 1900.

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Argentina—First National Conference on Neglected and Delinquent Children

THE first national conference on neglected and delinquent children in Argentina was held in Buenos Aires from September 25 to September 30, 1933. It was organized by the National Council for the Welfare of Minors (Patronato Nacional de Menores), a government agency established in 1931 for dealing with the welfare of neglected and delinquent children. The conference was attended by nearly 100 delegates from various parts of the country. The delegates urged the establishment of one-judge juvenile courts throughout the country and the enactment of uniform child welfare legislation in the various provinces. A bill to carry out these provisions was presented at the conference. It was decided to hold such conferences every two years.—*La Prensa,* Buenos Aires, Sept. 25–Oct. 1, 1933.